

Growth and rooting of *Solanecio mannii*: comparison of seedlings and air layers on a 24-month trial in East Africa

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Abstract: Vegetative propagation of trees remains understudied in Africa. Such methods however provide potential for producing trees and shrubs with high social and economic value. Air layering is one of these techniques and can be used in Uganda for the domestication of underutilized multipurpose trees. The main purposes of this preliminary trial, carried on small scale and without repetition, were to compare the growth rates of 30 *Solanecio mannii* plants grown from seed and 30 plants grown from air layering, and to observe the main morphological differences that affected the growth and root system. This 24-month prospective trial compared the growth characteristics of seedlings and air layers, by measuring their height, diameter, precocity to flower and fruit, and their root system quality. Air layers were stronger, quickly reaching maturity but showing weakness in their root systems, while seedlings seemingly smaller and slower showed more stability with a well-structured root system. Air layering could be used to reproduce desirable genotypes, but a more rigorous experiment with adapted statistical design should be programmed over minimum 5 years, because the relative data with this kind of test are excessively rare in the international bibliography.

Keywords: vegetative propagation, air layering, true seedling, root system.

Introduction

Over the past decade in North and West Africa, scientists have sought potential in the vegetative propagation of trees, especially by root suckering (Harivel *et al.*, 2006; Ouedraogo *et al.*, 2006; Belem *et al.*, 2008; Bognounou *et al.*, 2009; Ouedraogo *et al.*,

2012; Fawa *et al.*, 2015), air layering (Kengue *et al.*, 2002; Anegbeh *et al.*, 2005; Asaah *et al.*, 2010, 2012; Noubissié-Tchiagam *et al.*, 2011; Bellefontaine *et al.*, 2013-b; Moupela *et al.*, 2013; Ofori *et al.*, 2015), and root cuttings (Houehouna *et al.*, 2009; Ky-Dembélé *et al.*, 2010; Mapongmetsem *et al.*, 2012; Sanoussi *et al.*, 2012; Hougnon, 2014), yet unequal commitment has been recorded in East and South Africa (Bellefontaine, 2005) where few researchers have studied such potential (Meunier *et al.*, 2008-a,b, 2010; Morin *et al.*, 2010; Mwang'ingo *et al.*, 2010, 2011).

Based on a study conducted on 122 species in Budongo forest (Mwaru *et al.*, 2008), 119 were reported as likely to develop stump shoots, but only one (*Oncoba crepiniana* De Wild. & T. Durand) produced root suckers. This underestimation shows that natural propagation by suckers or layers remains poorly considered by foresters and botanists, despite the fact that it exists and represents high prospects for plant propagation and domestication. In 2008, Meunier *et al.* (2008-a) discovered that out of 30 forest species studied, 6 produced root suckers, 20 were successfully propagated by stem cuttings, 9 by root cuttings, 12 by terrestrial layering, and 15 by air layering. These study results are one of the outcomes of a five-year project started in 2005, promoting the reintroduction of indigenous woody resources in Bushenyi District, Uganda, with the aim of providing rural communities with multipurpose indigenous trees and shrubs (Meunier *et al.*, 2008-b). The western part of the country is one of the most inhabited regions in East Africa. Farming and domestic animal rearing represent the leading activities in the region, and thus for decades have induced high pressure on natural ecosystems, especially woody resources. However, wood remains the cornerstone of communities' livelihoods as they daily rely on it for firewood and charcoal, as well as medicine, fruits, fodder, construction or fencing.

In order to increase tree planting in agrosystems, various propagation techniques were developed, including propagation by seeds, but also vegetative propagation methods, such as layering, cuttings and suckering. The latter methods provide numerous advantages commonly mentioned in the literature (Hartmann *et al.* 2002), such as a faster growing rate and earlier maturation. There is strong evidence of this for the first months of the plants' life, but few cases suggest scientific records of this statement on a longer time scale.

This case study shows the results of an exploratory plot (without replication) for the species *Solanecio mannii* (Hook.f.) C. Jeffrey (Asteraceae). The main purpose of this preliminary study was to compare the growth rates and characteristics of 30 plants grown from seeds and 30 plants grown from air layers, through monthly monitoring of the plot over a 24-month period.

The study tested the following hypothesis: results after two years of growth will reveal whether individuals originating from air layering grow faster with a well-balanced root system and/or reach an earlier mature stage.

Material and methods

Species description

Solanecio mannii is a fast-growing woody shrub or a small tree, up to 10 m in height. Its architecture is related to the Leeuwenberg model (Hallé, 2004); in the first stages it grows into a straight monostem before branching into three, four, or rarely five stems, each one repeating this growth mode. The flowering and fruiting appear after each branching, the inflorescence shooting at the branch base. In Western Uganda, this species is used especially to cure poliomyelitis threats, and is highly sought by traditional healers. Its soft wood is not much valuable, but the species is appreciated for live fencing and boundary marking (Katende *et al.*, 1995; Meunier *et al.*, 2010).

Regarding its propagation potential, recent studies (Meunier *et al.*, 2008-a) show a high capacity for this species to be propagated using stem cutting, as well as air layering and terrestrial layering methods. Wildings are commonly found and collected nearby mother trees.

Plant selection and experiment outline

In order to compare growth and root-system development, we used 60 plants: 30 grown from seeds and 30 from air layers, in an exploratory plot. Although the species shows the ability to be propagated by stem cuttings, we did not include the latter in this trial. Germinating seedlings (30) grown in March 2007 were collected under and around several mother trees. Organized in a small thicket, the seedlings were immediately and carefully transplanted into plastic pots. The pot's substratum was made of soil and sand in an equal proportion. After five months of nursery care the seedlings were carefully removed from their plastic pots and planted in the experimental plot, in August.

As regards the air layers, two were placed on 15 young mother trees, on straight main branches of 1 to 2.5 cm in diameter. A 3cm-long injury (bark removal) was made all around the stem and covered with a substratum comprising 80% moss, 10% soil and 10% sawdust (without hormones). The 30 air layers were made in mid-June, beginning of the dry season, and all successfully rooted (at least $\frac{3}{4}$ of the plastic bag was filled with roots) (Fig. 1) and were planted, without pruning or pollarding, in August 2007.

At the starting point of the experiment thus prevailed an age difference between the plants grown from seed (five months) and the plants grown from air layers (two months). Seedlings and air-layer stems were intermediately placed in an 80 m² plot, arranged in five lines each bearing six individuals. The soil was even in terms of



Figure 1- Rooted air layer of *Solanecio mannii*.

topography, as well as the ground cover and canopy composition (comprising 20-year-old *Grevillea robusta* A.Cunn. ex R.Br. exclusively). In order to avoid inter-individual competition, all plants were spaced 2m apart. Finally, during the 24-month experiment three air layers and two seedlings were totally or partially destroyed due to several sporadically fallen branches from the *G. robusta* canopy. They do not appear in the results.

Plant measurements

The plot was set up in mid-August 2007, and measurements were made every month. Recorded parameters for each individual were:

- height, from the base of the seedling up to the highest terminal leaf bud;
- diameter, at the plant base (5 cm above ground level);
- architecture, accounting for plant maturity, using the number of branching points;
- flowering and fruiting periods.

During measurement, a monthly and uniform cleaning of young new plants was operated on the plot to avoid climbers' disturbance or inter-species competition.

Final uprooting, root system measurement and plant health appreciation

After 24 months, all 60 plants were removed (full excavation). This operation was carried out by carefully digging all around the plants to avoid damaging the root systems. Root systems were lightly watered to help remove soil and untangle the roots.

Their picture was then taken and they were measured, noting the following characteristics:

- root quantity (qualified as “few” or “abundant”)
- root-system structure (qualified as “poorly balanced” or “well-balanced”)

For the last 5 months of the experiment (April to August 2009) the plot had been exposed to an unusual severe drought period. Many individuals suffered from lack of water, resulting in various growth deficiencies that were clearly visible, and individuals were classified into three categories: (i) healthy, (ii) suffering and (iii) dying. Concurrently with this health qualification, all plants were thus uprooted and classified into 4 categories: (i) abundant and well-balanced, (ii) abundant and poorly balanced, (iii) few and well-balanced, (iv) few and poorly balanced.

Results

Heights

In August 2007, on day 0, the average height of the air layers was 58.5 ± 19.9 cm for an average seedling height of 16.1 ± 5.7 cm, thus a differential of 42.4 cm. This difference regularly increased to reach a maximum of 109.2 cm in month 12. This value then slightly reduced and oscillated around 100cm for the following 6 months, and then reduced and stabilized around 60cm from months 20 to 24. The total average height increment was 324 cm for seedlings and 344 cm for air layers (Fig. 2). The air layers grew slightly faster at the beginning and kept their height and strength advantage throughout the experiment. In terms of growth, the air layers developed their original size about 7-fold, while the seedlings increased by over 20 times compared with their original size. Air layers were higher and stronger, yet height growth was more intensive for seedlings.

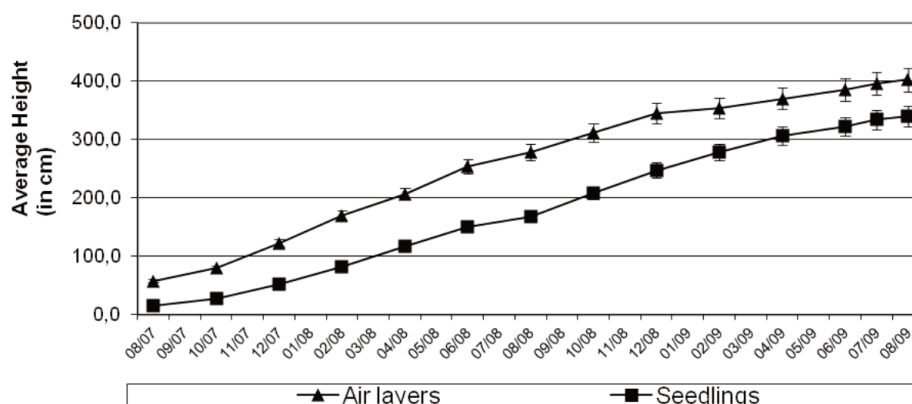


Figure 2 - Height comparison of air layers and seedlings.

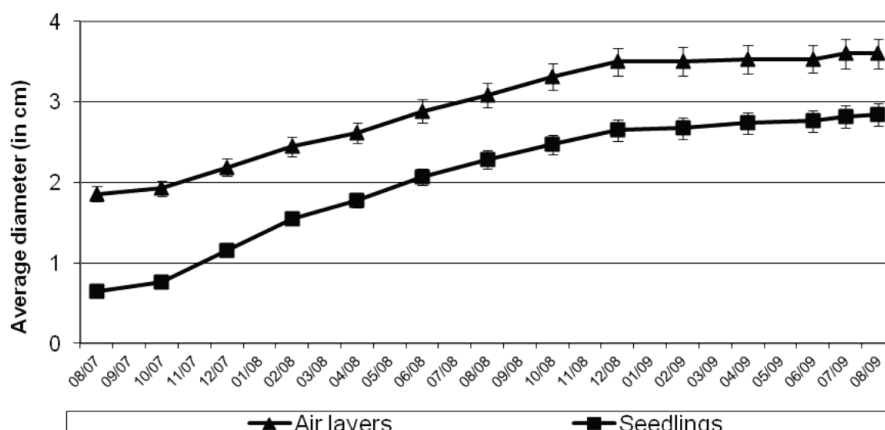


Figure 3 - Diameter comparison of air layers and seedlings.

Diameters

In August 2007, on day 0, the air layers showed an average diameter of 1.8 ± 0.4 cm, while the seedlings showed an average diameter of 0.6 ± 0.2 cm, thus a differential of 1.2 cm. This difference gradually decreased for the first 10 months, to reach a value of 0.8 cm, a difference which remained unchanged until August 2009. The total average diameter increment was 2.2 cm for seedlings and 1.7 cm for air layers (Fig. 3). Average diameter of air layers increased about 2-fold compared with its original size, while the seedlings' diameter increased by more than 4.5 times.

Architecture

Architecture was compared using the number of branching points for each individual (Table 1; Fig. 4). At the starting point of the experiment, six air layers already bore a branching point (they actually branched during the month of air-layering settlement). The first branching to occur on the experimental plot was recorded after one month for the air layers and after 10 months for the seedlings. 100% of air-layer individuals branched once before month 10 and twice before month 18; 85% branched a third time and some individuals reached a fourth and fifth branching in August 2009 after 24 months. On the other hand, 82% of the seedlings branched a first time, and only 6% of them branched twice.

Fruiting periods

After 24 months, all air layers produced fruits at least once, with the fastest maturation occurring only 4 months after planting (Table 2). The average period of

Table 1 - Structure comparison: number of branching points for each individual during the 24-month trial.

NUMBER OF BRANCHING POINTS	SEEDLINGS (%)	AIR LAYERS (%)
1 st	82	100
2 nd	6	100
3 rd	0	85
4 th	0	37
5 th	0	4

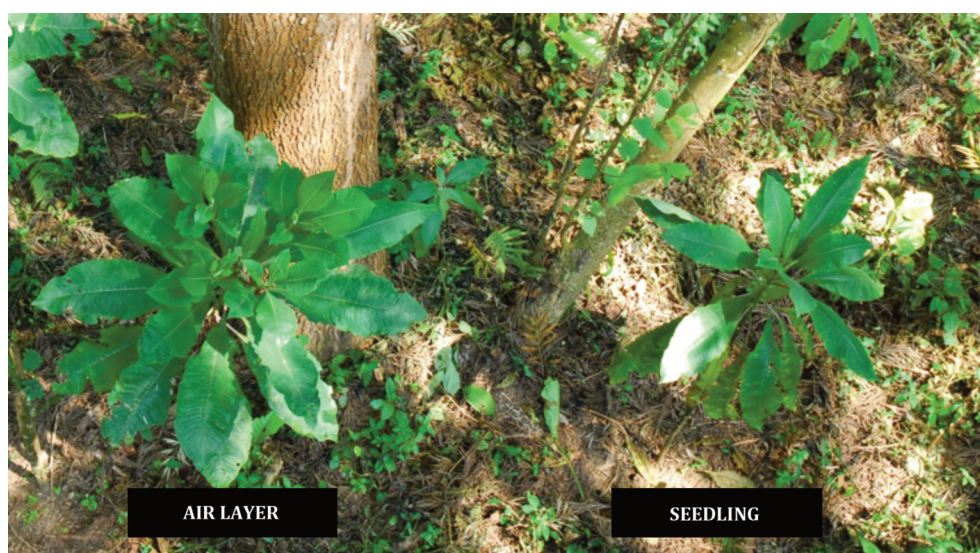


Figure 4 - Structure comparison of an air layer and a seedling.

time for maturation is 10 to 11 months. Moreover, 96% of individuals produced twice, and 67% thrice. As regards seedlings, only 13 of them (46%) reached maturity after 18 to 24 months. 11% fructified a second time and, after 24 months, 15 of them; hence over 50% of all individuals remained immature (Tables 2 and 3).

Root-system comparison and health appreciation

Table 4 displays results of the root-system comparison. Plants grown from seed in most cases presented a good-quality root system as for more than half of the population roots were abundant and well-balanced, while less than a third of air layers comprised these two characteristics. In parallel, seedlings were healthier than air layers with 23 healthy seedlings versus 5 air layers.

Such morphological disparities between seedlings and air layers are illustrated (Fig. 5), in order to highlight the different root architectures that influence plant growth and stability.

Table 2 - Maturity comparison of seedlings (28) and air layers (27).

FRUCTIFICATION NUMBER	SEEDLINGS (28)	AIR LAYERS (27)
1	13 (46%)	27 (100%)
2	3 (11%)	26 (96%)
3	0 (0%)	18 (67%)

Table 3 - Fructification time-period comparison of seedlings (28) and air layers (27).

FRUCTIFICATION TIME-PERIOD	SEEDLINGS (28)			AIR LAYERS (27)		
	1 ST FRUITING	2 ND FRUITING	3 RD FRUITING	1 ST FRUITING	2 ND FRUITING	3 RD FRUITING
0-6 months	-	-	-	4%	-	-
6-12 months	-	-	-	78%	-	-
12-18 months	8%	-	-	18%	74%	-
18-24 months	92%	100%	-	0	26%	100%

Table 4 - Root-system comparison of seedlings and air layers after 24 months.

ROOT SYSTEM	SEEDLINGS (28)			AIR LAYERS (27)		
	HEALTHY (82%)	SUFFERING (11%)	DYING (7%)	HEALTHY (19%)	SUFFERING (22%)	DYING (59%)
Abundant and well-balanced	17	1		3		5
Abundant and poorly balanced				2	6	2
Few and well-balanced	6	1	2			1
Few and poorly balanced		1				8



Figure 5 - Root-system organization for a seedling (left) and an air layer (right).

Discussion

In facing woody species' domestication and conservation challenges one should consider the potential of vegetative propagation, and particularly the air layering method, which is very cheap to carry out, easy to handle and quite successful (Meunier *et al.*, 2008-a; Asaah *et al.*, 2012; Bellefontaine *et al.*, 2013-b). In this preliminary and exploratory trial, results obtained for growth comparison of seedlings and air layers revealed three main characteristics:

- air layers grew stronger but not faster. Plants originating from vegetative propagation reportedly grow faster, and this was only partially confirmed here. In a South African trial with *Uapaca kirkiana* derived from grafts, marcots and saplings, tree height, root-collar diameter, crown depth and crown spread of marcots were better as compared with the same characteristics for *Uapaca* saplings, eight years after planting (Dawson *et al.*, 2011). While it was noticeable that it conserved its stronger habit and its diameter and height advantage throughout the experiment, it however developed in average about 7-fold compared with its original size, and the seedlings increased by over 20 times compared with their original size. The “energy” required for growth was much higher for seedlings. This great difference could probably be linked to the fruiting precocity of the air layers (Hartmann *et al.*, 2002). According to Moupela *et al.* (2013), air layering produced far more vigorous *Coula edulis* plants within shorter time than nursery-raised seedlings.
- maturity was reached significantly faster for air layers. Since air layers derived from older vegetative material, thus nearer to maturity, the “energy” saved in growth could have been directed towards reproduction, resulting in high productivity, as all air layers produced three times while some seedlings never reached maturity during this 24-month trial. This fact has an important social and economical value, as this potential could for example be used for medicinal-plant and fruit-tree

production, rapidly and quantitatively meeting local demands, which are vital pleas in parts of tropical Africa. Eight years after planting, fruit weight was 13.0 +/- 0.35g for marcots versus 0g for saplings (Dawson *et al.*, 2011).

- a non-structured, rather anarchic root system for air layers compared with seedlings. Although air layers are promising, their root system's scantiness is to be considered. In our experiment, this was most probably the cause of deaths and weaknesses observed during the dry period. Adventitious roots from the air layers did not colonize deep enough underground and were too poorly structured, which resulted in vulnerability to droughts and liability to fall down. This gap regarding the root-system quality could also be the cause of a reduced growth speed, compared with seedlings. Yet, such poor root-system establishment for air layers has not always been recorded. In West Africa, Ofori *et al.* (2015) showed that for *Allanblackia parviflora* "the success of rooting of layers declined with increasing height of pollarding; Juvenile gradient has been observed in trees from base to the top". In our experiment, would the root systems of *Solanecio mannii* have been more vigorous if the air layers had been initiated more closely to the root collar? According to Mwang'ingo *et al.* (2010), air layers initiated in June and September during the dormant phase (dry season) in Tanzania had better rooting compared to those of December and February (rainy season). But it is very important to note that Asaah (2012) mentions that "*Dacryodes edulis* trees of vegetative origin (air layers) have well-developed adventitious primary root system and deep sinker roots". In India, the juvenile plants of *Drypetes malabarica* have resulted 100% rooting success for air layering and the rooted plants were also survived with 100% success rate (Jose *et al.*, 2015). Further investigations are needed to find the main (internal and/or external) factors influencing root development of air layers.

Comparisons of seedling and air-layer root systems are very poorly documented. According to Asaah *et al.* (2010), tree propagation methods can influence the root system of trees. *Dacryodes edulis* (G. Don) H.J. Lam. trees of vegetative origin had a wider and deeper root spread compared with trees of seed origin; the former developed dominant vertical roots which could be an important adaptation due to the absence of a taproot. For the Argan tree, cuttings showed the same root architecture with 4 or 5 dominant vertical roots and a very good survival after one year (Bellefontaine *et al.*, 2013-a, b). Fine root distribution of *D. edulis* trees of seed and vegetative origin varied according to depth and lateral distance from the tree base, and trees of seed origin showed significantly higher density in the 0-30cm soil stratum compared with *D. edulis* trees of vegetative origin (Asaah *et al.*, 2012). In Central India, the growth of air-layered *Madhuca latifolia* Roxb. is better after 12 years when compared to Indian published literature on seedling originated *M. latifolia* (Chavan *et al.*, 2015). The use of auxins improved the survival and architecture of the root system (Mapongmetsem *et al.*, 2012; Duran-Casas *et al.*, 2013; Ramirez-Malagon *et*

al., 2014; Dako *et al.*, 2014; Yeboah *et al.*, 2014; Cassol *et al.*, 2015; Chavan *et al.*, 2015), but the purpose of this “trial” was to present a low-cost and efficient method which can be used by farmers in rural communities.

A wind throw tendency was previously observed (Kengue *et al.*, 2002), and nursery techniques should be tested to avoid such adversities as they can have a devastating impact when considering a large-scale plantation of successful air layers, in terms of commercial purposes. Maintaining successful air layers in tall pots, out of ground in order to facilitate the development of main adventitious roots similar to taproots and to reduce side roots could indeed be a solution (Bellefontaine *et al.*, 2012).

In addition to this exploratory trial, it would be appropriate to adopt a statistical design for the next test between air layers and saplings. Alternatively, air layers could be grown in order to select *ex situ* high-value genotypes that could then be used for grafting or cuttings.

Conclusion

Air layers and seedlings grow significantly differently. Air layers made on a vertical sprout with an adapted substratum have many viable roots and appear healthier and stronger, reaching their mature stage very rapidly. However, in our plot their root system remains small and poorly balanced after two years, compared with true seedlings. As a consequence, plants grown from air layers are more sensitive to environmental factors, such as wind or drought. Seedlings are slower in terms of establishment and much less suitable regarding the time needed to reach maturity, but are well-structured and resistant.

Air layering could be used particularly to reproduce desirable genotypes. After harvest from the mother tree, transplantation and care in a nursery, air layers are very useful for the initial collection of elite and old genotypes.

Such perspectives require additional research in order to combine most advantages in one plant material that can meet the socio-economic context in tropical Africa: rapidly acquiring quality plants for food, medicine, firewood and construction. These perspectives offer new insights into the domestication of *Solanecio mannii* and others agroforestry trees.

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